Influence of probiotic on serum biochemical profile,

Growth performance and immunostatus in broiler chickens.

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Abstract.

A total of 90 one-day-old male broiler chicks were fed on balanced ration for 15 days, then divided into 3 equal groups. Control group fed ration probiotic-free, groups 2 and 3 fed ration supplemented with probiotic at concentration levels 0.5 and 1.0g/kg ration respectively for 42 days. It was found that probiotics improved body weight gain and feed conversion ratio. Relative organs weights were not altered in groups of birds fed ration mixed with probiotic. Serum values of biochemical parameter (AST, ALT, ALP, total protein, A/G ratio, Ph) were not significantly changed in comparison to control group. Probiotics enhanced the immunostatus of chicks in a dose dependent relation as documented by increasing the serum antibody geometric titers of ND vaccinated chicks and phagocyte percentage and phagocytic index of peripheral blood monocyte of broiler chicks. We concluded that the probiotic (Gro2Max)* enhanced body weight gain, feed conversion, growth performance and immunostatus of broiler chicks.

Introduction

Antimicrobials have been used as a supplement for more than 50 years in poultry feed, to enhance growth performance and to prevent diseases in poultry. However, in recent years great concern has arisen about the use of antibiotics as a supplement at sub-therapeutic level in poultry feed. Due to the emergence of multiple drug resistant bacteria **(Wray and davies, 2000)** antibiotics as a feed supplement can be replaced by alternatives such as a probiotic.

Probiotic are live non-pathogenic microorganisms that when administered in adequate amount exert benefits to host health and the immune system through an improvement in the intestine, as microbial equilibrium **(Fuller, 1989; Reid et al., 2003; Gunal, et al., 2006 and**

Vinderola et al., 2006). Therefore, they are called friendly bacteria. They cooperatively maintain a delicate balance between the gastro-intestinal tract and immune system. When this balance is disrupted, disease and inflammation results. Inflammation and overstimulation of the immune system by pathogenic bacteria are competitively inhibited by mucosal adherence of normal beneficial microflora **(Wysocka, 2001)**. However, the role of different types of probiotics on health performance, serum biochemical changes and immunostatus of male broiler chicks is still not fully clear.

Therefore, the purpose of this study was to evaluate the effect of a new probiotic formula (Gro2Max) in improving the performance, body weight gain, feed conversation, and immunostatus of broiler chicks.

Materials and Methods

Probiotic:

Probiotic (Gro2Max)* is obtained from BioNatural America Institute in the form of a non-soluble feed additive powder composed of five strains of bacteria ***(Bacillus subtilis, Lactobacillus acidophilus, Pediococcus acidilactici, Pediococcus pentosaceus & Saccharomyces cerevisiae)***. It was added to the ration in two concentration levels 0.5 or 1 g/kg ration for four successive weeks.

Experimental design:

A total of 90 one-day-old male Hubbard broiler chicks were fed on balanced ration and water ad libitum for 15 days, then divided in a randomized complete design into equal groups of 30 birds each.

Group 1 fed ration probiotic free.

Group 2 fed ration supplemented with probiotic 0.5 gram/kg ration.

Group 3 fed ration supplemented with probiotic 1 g/kg ration.

Ration:

A basal ration was formulated and considered as control according to recommendation of NRC (1994) for starter (0-21 days crude protein 21%) and grower (22-42 days, crude protein 18.5%).

All birds were reared on floor pens for the end of the experiment (42 days).

Body weight gain and feed conversion ratio were recorded weekly. Birds were slaughtered at the end of the experiment for weighing of carcasses, feather & skin, liver, gizzard, heart and spleen.

Blood sampling:

1. **For biochemical studies:**

At 2nd day of the experimental period 5ml of blood was collected from wing vein from birds in each group. Blood samples were centrifuged (at 3000 x rpm for 10 min) and serum was separated and stored at 20°C until assayed for using appropriate laboratory kits [AST, ALT and ALP **(Tietz, 1999)**; total protein and albumin **(Young, 1995)**]. The serum globulin was calculated by subtracting serum albumin from serum total protein levels and A/G ratio. Creatinine was determined according to **Bartels (1961)** and calcium and phosphorus according to **Tietz (1995)**.

2. **For immunological status studies:**

Blood samples were collected from all groups of birds every ten days post vaccination for serum separation to determined antibody against Newcastle diseases virus (NDV) vaccine by haemagglutination inhibition (HI). Hepranized blood samples were collected at 1st day post the booster dose to determine the phagocytic activities.

a. Haemagglutination inhibition test (HI):

The serum samples were utilized for HI test to find out the level of antibody titer developed against NDV vaccine in different groups of birds. The test was carried out according to **King and seal (1998).**

b. Phagocytosis:

Measurement of phagocytic activity of peripheral blood monocytes using Candidia albicans by separation of peripheral blood mononuclear cells (as described by **Boyum, 1968)**. Mononuclear cell layer was collected, washed and resuspended in RPMI-1640 supplemented with 10% foetal calf serum and viability was done after **Hanks and Wallace (1985).** The test was performed according to procedure described by **Chu and Dietert (1989).**

Stastistical analysis:

Data were an analyzed using the one way ANOVA procedure according to **Petrie and Watson (1999)** and **(Snedecor, 1969).**

Results and Discussion

The results obtained from this study revealed that ration supplemented with probiotic at concentration 0.5 or 1 g/kg ration improve body weight gain in comparison to control group of birds (Tables, 1 & 2 and fig. 1). Feed conversion rate was improved in group of chicks fed ration mixed with probiotic 0.5 g/kg ration (2.5) than either birds fed 1g probiotic /kg ration or control group of birds fed ration free probiotic (3.3 and 3.4 respectively) (Table 3 & fig. 2). The improvement of body weight gain and feed conversion in group of birds fed ration supplemented with probiotic might be due to improved intestinal microbial balance, reduced pathogenic flora which accelerated food absorption **(Swain *etal.*, 1996).** Probiotic may stimulate appetite and improve nutrition by the production of digestible enzymes (phytase, amaylase, sucrose, protease, cellulose and lignase) and vitamins, detoxification of injurious compounds in the diet and by the breakdown of indigestible compound **(Pelicano *et al.*, 2003).**

The results of the present study substantiated the findings of **Thitaram *et al.* (2005); Nayebpor *et al.* (2007) and Ashayerizadeh *et al.* (2009)** who reported that probiotics and prebiotics can improve the weight of birds. Moreover, adding probiotic and symbiotic to the ration has been effective in improving the feed conversion ratio **(Zulkifli *et al.*, 2000 and Cavit, 2004),** Which is contrary to the report of **Gunal *et al.* (2006), Willis *et al.* (2007)** and **Yalcinkayal *et al.*, (2008)** who reported

that using this additives shed in the broiler ration had no significant effects on body weight gain and feed conversion ratio after experimental period of 42 days.

For safety evaluation of probiotic as a fed additive, it was found that relative organ weight (liver, gizzard, heart and spleen) was within the normal weight without any significant changes in comparison to control group of birds (Table 4). This indicates that probiotic induces no side effect to groups 2 or 3 of chicks. This finding is in agreement with **Mohan *et al., (1996)*** and **Islam *et al.* (2004)** who found that supplementation of probiotics had no significant effect on relative organs weights. While the relative carcass weight of group of birds fed ration mixed with probiotic (0.5g/kg ration) increased significantly ($P<0.01$).

Moreover, the biochemical parameter (total protein, albumin, ALT, AST, ALP, creatinine and phosphorus) was significantly not altered by the probiotic addition to the ration of chicks (Table, 5). Similarly, **Midilli *et al.* (2004)** observed that feeding of probiotic did not affect the serum total protein in the treated groups of broilers. Also **Rangasamy and Kalippan (2007)** reported that the serum ALP, AST, ALT and total protein levels in probiotic broiler fed groups did not differ significantly from control group.

Globulin and A/G ratio was increased significantly in group of birds fed ration mixed with probiotic 1g/kg ration. This indicates that probiotic at this concentration enhances globulin formation which indicates higher defensive mechanism of the birds. Theses results were assured by immunological studies which proved by the immunostatus of broilers. Probiotics stimulate production of natural antibodies in chickens **(Hamid *et al.*, 2006).** However, serum calcium concentration was decreased significantly ($p<0.01$) in group of birds fed ration mixed with probiotic at 1g/kg ration in comparison to control group but still within normal serum calcium level of broiler (Table, 5) without any adverse effect of hypocalcemia. This result was in agreement with **Antunovic *et al.* (2005)**. They found that serum calcium level decreased significantly ($P< 0.01$) in lamb fed ration mixed with probiotic for 35 days.

Probiotics confer immune adjuvant properties to the host when administered in appropriate dose. The ability of probiotics to prevent

several pathological conditions was by stimulating the immune system to generate an immune response to be able to interact or send signals to immune cells associated with the gut **(Isolauri *et al.*, 2001).**

Serum antibody geometric mean titers against NDV are shown in Table (6). The HI titer against NDV was higher with groups 2 and 3 of broiler fed 0.5g and 1g probiotics/kg ration respectively. While, the control group which was fed basal diet (Group 1) recorded the lower titre. The level of immune titre against NDV increased continuously up to 30 days post vaccination in the two probiotics treated groups compared to the control one. **Rangasamy and Kaliappan (2007)** indicating immunomodulatory effect of probiotics in broilers, and a higher HI titre against NDV in probiotic treated groups while, the control showed low titre, additionally **Kabir *et al.* (2004)** found that supplementation of probiotics significantly increased the antibody production in broilers.

Referring to phagocytic activity of monocytes (Table, 7), a significant increase was recorded in both phagocytic percentage and index in treated group. Probiotic bacteria contact was mucosal and systemic adjuvant, inducing an interaction of probiotic bacteria with the immune cells of payer's patches from the innate immune system, therefore cytokines production is enhanced. These cytokines are the biological messengers of signals that activate the systemic immune response **(Galdeano *et al.*, 2007)**. Also, **Schiffrin *et al., (1997)*** stated that a bacterial signal could also act directly on epithelial cells or the intraepithelial lymphoid compartment of the proximal small intestine. This lymphoid population, on activation, can produce cytokines, such as interferon γ and interleukin 2 **(Ebert, 1989 and 1990)**. Cytokines released from these lymphoid populations may then act locally or alter the systemic reactivity of the host. It is tempting to speculate that for some probiotic activities such as immunomodulation or competition with some pathogens the host target of LAB is localized in the proximal gastrointestinal tract, whereas for other pathogens, ie, Shigella ssp., the target is localized in the colon.

LAB (lactic acid bacteria) and their products can induce in vitro interferon γ production by human lymphocytes and interferon α production by peritoneal macrophages **(Matsumura *et al.*, 1992 and Solis and Lemonnier, 1993)**. In addition, LAB products given orally can

induce endogenous tumor necrosis factor α production in mice **(Davidkova *et al*., 1992)** and also enhance other macrophage and neutrophil functions. Moreover, LAB products can alter macrophage functions in vitro with a species-dependent pattern as shown by **Hatcher and Lambretch (1993).** A significant increase in phagocytic percentage and index in group 2 and 3 compared to group 1 (Table 7) proved that the immunomodulation pattern depends on the bacterial dose. **Also, Jessus *et al.* (2002)**, who worked on yeast, stated that activation mechanisms involved are known to be related to the carbohydrates derived from the yeast cell wall, and β-glucans added to the feed stimulated the phagocytic function. Not only sugars but also nucleic acids, especially yeast RNA, could act as immune activators, since the growing evidence that nucleic acids from yeast sources, previously considered nutritionally non-essential for immune reactivity in mammals.

In conclusion, dietary supplementation of probiotic for male broiler chicks significantly increased body weight gain, feed conversion ratio and improve immunostatus. Moreover, probiotic was safe as proved by serum biochemical profile and relative organ weight of male broiler chicks. In veterinary medicine probiotics may be effectively used to optimize digestive processes, stimulate growth and prevent diseases of the digestive tract.

Table (1): Body weight (g) of male broiler chicks fed ration mixed with probiotic at different concentration levels (0.0, 05 and 1 g/kg balanced ration) (mean ± S.E.) n=30.

Group	Dose (g/kgration)	Date				
		2nd week	3rd week	4th week	5th week	6th week
1st group	0.0	333.8±7.03	753±21.67	1225.17±26.27	1792.67±41.75	1917.17±47.63
2nd group	0.5	362.73±6.67**	787.667±14.97	1309.5±22.11*	1902.5±31.25*	2270±40.11***
3rd group	1	362.83±5.83**	771.5±15.78	1267.5±22.20	1798±36.84	2137±53.02**

Significant at *P<0.05 P<0.01 ***P<0.001**

Table (2): Body weight gain of male broiler chicks fed ration mixed with probiotic at different concentration levels (0.0, 0.5 and 1g/kg balanced ration) for 4 successive weeks (mean ± S.E.) n=30.

Group	Dose (g/kg ration)	3rd-2nd week	4th-3rd week	5th-4rd week	6th -5th week
1st group	0.0	419.2±17.1	472.17±9.61	570.83±15.99	251.72±10.22
2nd group	0.5	424.93±11.62	521.83±11.99**	593.62±17.72	387.14±1438***
3rd group	1	408.67±11.84	496.0±9.895	528.1±17.51	353.45±26.19**

Significant at ** P<0.01 *** P<0.001

Table (3): Mean of body weight gain, mean of ration consumed and mean of feed conversion ratio of male broiler chicks fed ration mixed with probiotic at different concentration levels (0.0, 05 and 1g/kg ration) for 4 successive weeks (Mean ± S.E.) n = 30.

Group	Dose (g/kg ration)	Mean of body wt. gain	Feed conversion	Mean of total ration consumed (kg)
1st group	0.0	1604.24±53.41	3.4	5416
2nd group	0.5	1947.83±68.87***	3	5833
3rd group	1	1697.41±80.76	3.2	5416

Significant at ** P<0.01 *** P<0.001

Table (4): Relative organ weights of male broiler chicks fed balanced ration mixed with probiotic at different concentration levels (0.0, 0.5 and 1.0g/kg ration) at time of slaughter (42 days) (mean ± S.E.) n=30.

Groups	Dose g/kg ration	Feather & skin	Carcass	Liver	Gizzard	Heart	Spleen
1st group	0.0	12.51±0.34	63.23±0.76	3.006±0.14	1.819±0.06	0.472±0.02	0.19±0.04
2nd group	0.5	13.17±0.31	67.98±0.36***	2.744±0.09	2.044±0.08	0.532±0.02	0.176±0.01
3rd group	1	13.297±0.41	65.08±0.93	2.756±0.18	1.829±0.05	0.512±0.03	0.177±0.04

Significant at *** P < 0.001

Table (5): Biochemical parameters of male broiler chicks fed ration mixed with probiotic at different concentration levels (0.0, 0.5 and 1g/kg ration) for 4 successive weeks. (Mean ± S.E.) n=30.

Groups	Dose (g/kg ration)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	Creatinine (mg/dl)	Calcium (mg/dl)	Phosphors (mg/dl)	ALT (u/L)	AST (u/L)	ALP (u/L)
1st group	0.0	3.95±0.119	1.166±0.077	2.784±0.133	0.426±0.042	0.311±0.016	11.24±0.33	9.296±0.31	42.5±3.44	149.0±11.23	164.56±11.1
2nd group	0.5	3.7±0.23	1.27±0.19	2.55±0.175	0.458±0.042	0.366±0.029	10.88±0.08	8.176±0.4	39.25±3.136	146.0±1516	160.45±11.7
3rd group	1	4.4±0.22	1.02±029	3.382±0.2*	0.306±0.016*	0.317±0.015	9.92±0.04**	8.477±0.19	38.25±4.27	138.3±15.49	152.98±9.9

*** Significantly different from control at P < 0.05**

*** Significantly different from control at P < 0.01**

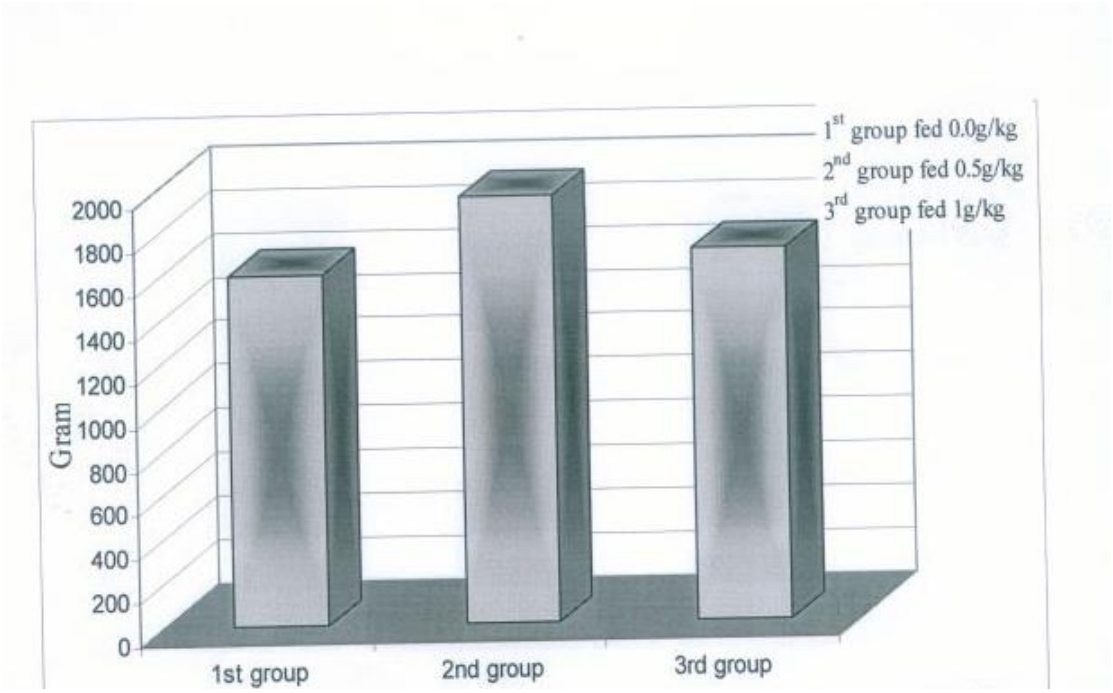
Table (6): Serum antibody geometric mean titres of ND vaccinated chicken by Haemagglutination inhibition (HI) test in male broiler chicks fed ration mixed with probiotic at different concentration levels (0.0, 0.05 and 0.1 g/kg ration).

Group	Dose (g/kgration)	Time		
		10 days	20 days	30 days
1st group	**0.0**	**49.324**	**89.808**	**123.286**
2nd group	**0.5**	**54.975**	**93.845**	**155.965**
3rd group	**1**	**52.293**	**90.166**	**146.979**

Table (7): Phagocytosis of peripheral blood monocyte of broiler chicks fed ration mixed with probiotic at different concentration levels (0.0, 0.5 and 1.0g/kg ration).

Groups	Dose (g/kg ration)	Parameter)	
		1 day p. booster dose of vaccine	
		Phagocytic percentage	Phagocytic index
1st group	0.0	54.00 ± 0.577^{c}	0.1300 ± 0.0100^{a}
2nd group	0.5	62.00 ± 1.154^{a}	0.2700 ± 0.0414^{a}
3rd group	1	73.00 ± 1.732^{b}	0.5033 ± 0.0639^{b}

Means with different superscripts in the same column are significantly different at $P<0.05$.


1st group fed 0.0g/kg
2nd group fed 0.5g/kg
3rd group fed 1g/kg
Gram
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400
200
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1st group
2nd group
3rd group

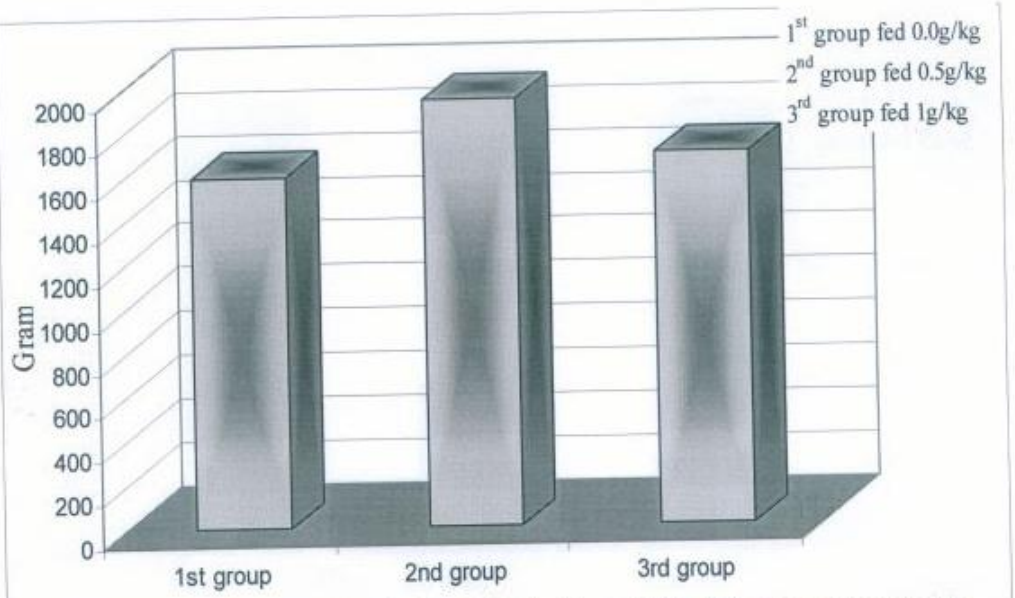


Fig. (1): Body weight gain for male broiler chicks fed ration mixed with probiotic at different concentration levels (0.0, 0.5 and 1g/kg ration) for 4 successive weeks.

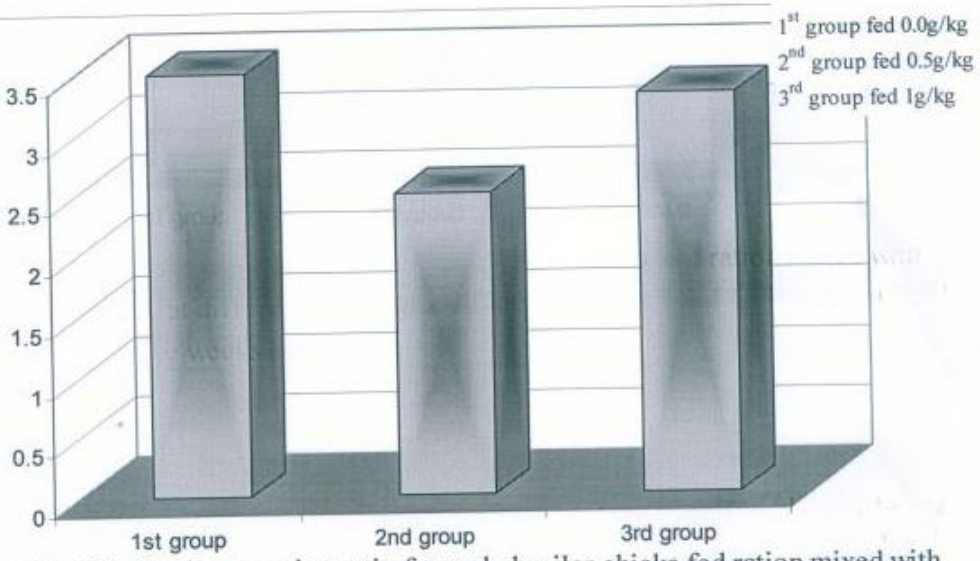
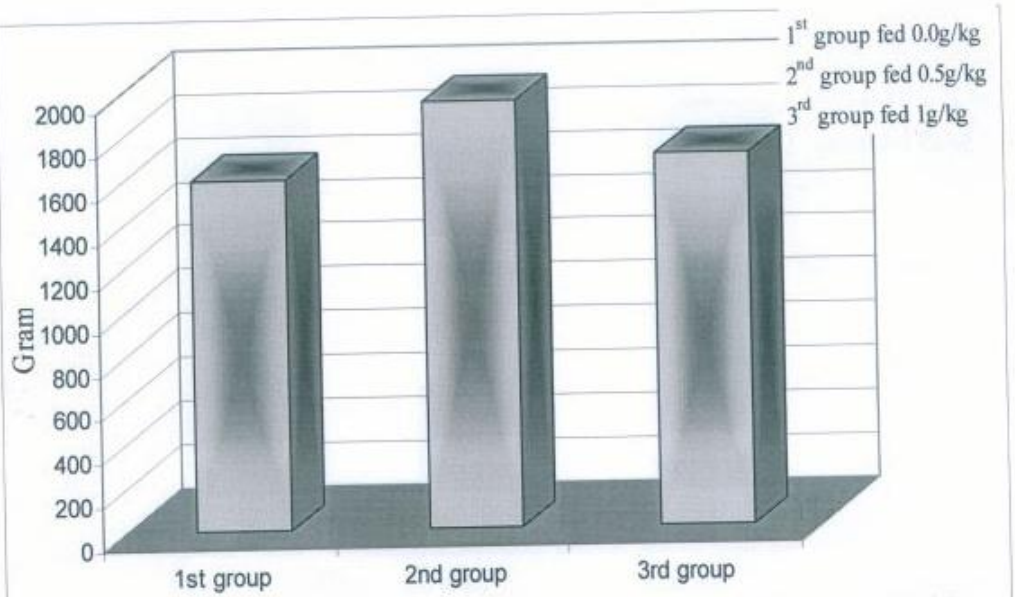


Fig. (2): Feed conversion ratio for male broiler chicks fed ration mixed with probiotic at different concentration levels (0.0, 0.5and 1g/kg ration) for 4 successive weeks.



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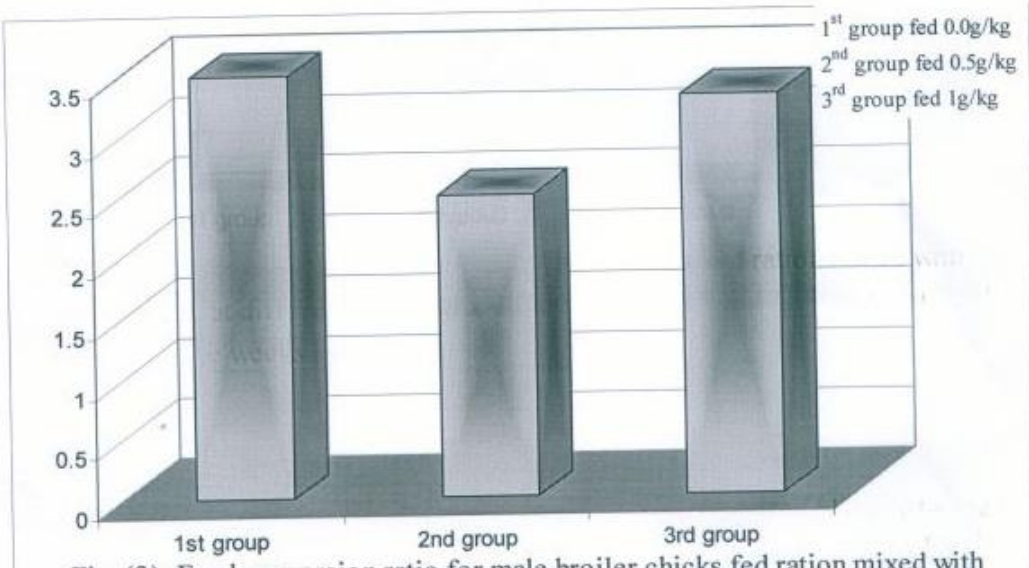

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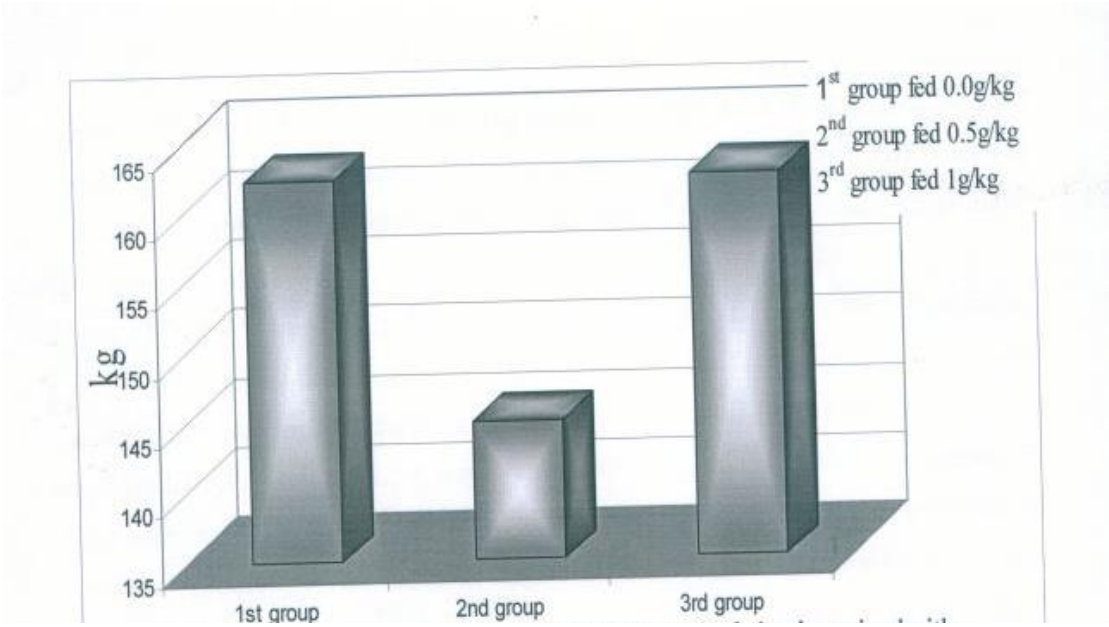

Fig. (3): Total ration consumed for male broiler chicks fed ration mixed with probiotic at different concentration levels (0.0, 0.5 and 1g/kg ration for 4 successive weeks.

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